A Study to Evaluate the Levels of Dioxin-Like Compounds in Dairy Feeds in the United States

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Introduction

The primary route for general population exposure to dioxin-like compounds is through the consumption of animal fats, with bovine-derived meat, milk and dairy products comprising over 50% of total exposure in the United States¹. The primary route of exposure hypothesized for cattle is airborne deposition of dioxins onto the leaves of feed crops. Over the last few years additional pathways of exposure have been identified associated with contaminated feed additives such as ball clay, mineral supplements, and animal byproducts. Studies by the United States Department of Agriculture (USDA) have shown that incidental contact with pentachlorophenol (PCP)-treated wood by cattle have resulted in elevated tissue levels. Although the air-to-leaf pathway is still considered by most researchers to be the dominant pathway of exposure, the lack of any systematic examination of animal feeds to quantify the contribution of the air-to-leaf pathway has been a major gap in our empirical understanding of dioxin exposure.

The Environmental Protection Agency (EPA), in cooperation with USDA and the US Food and Drug Administration (FDA), has undertaken a program to study the presence of dioxin-like compounds in animal feeds. Two phases of this program have been completed, and this paper reports on the third phase. The first phase was a study on the mass balance of dioxins in lactating cows^{2,3,4}. The objective of that study was to quantify the role feeds play in total dairy cow exposure. The second phase of the program involved the collection and measurement of dioxins in minor feed components⁵. Dioxins in specific targeted animal feed components of interest, including animal byproducts (beef, pork, poultry by-products, fish meal) and plant byproducts (deodorizer distillates from corn, soybean, peanut, cottonseed, and canola processers; cane and beet molasses), were measured. The

third phase of the project, reported here, involved component sampling of dairy feeds around the US.

Study Design

Dairy feeds are often classified as forages and concentrates. Forages are characterized by being more fibrous (higher than 30 percent neutral detergent fiber) or bulky and generally represent the vegetative portion of a plant. The major groups of forages include pasture and range plants, hays and silages. Concentrates include grains, grain by-products, oilseed meals, animal by-products, fruit and sugar processing by-products. Corn is the primary grain included in dairy feed and is fed in similar proportions in all regions of the country, with soybeans and cottonseed also primary components of dairy feed concentrate. In addition to forages and concentrates, minor components such as minerals, vitamins, animal fat additives and others, are also included in dairy feeds.

This study entailed the collection of the dairy feed total mixed ration, or TMR, forage components, concentrate components, and minor components at ten US government and state university research facilities which raise dairy cattle in a manner similar to commercial dairy operations. The purpose of this study is to determine the relative contribution of various feed components to the total dioxin content of dairy feeds. The selected research facilities are representative of several different regions of the county including the northeast, upper midwest, pacific and southeast. Figure 1 shows a map of the US identifying the general locations of the facilities. Table 1 provides a list of the components sampled in each facility, and the percentage that each component contributes to TMR. Not listed on this table are 2 samples of TMR collected at each location, as well as one composite sample each of milk and feces from lactating cows.

The facilities were sampled between April, 2002 and January, 2003. EPA traveled to each facility, and over the course of a few days, collected, boxed and shipped the samples to the USDA Dairy Forage Research Center (DFRC) in Prairie du Sac, Wisconsin. At the DFRC, samples were refrigerated until being dried and ground to a fine powder in preparation for their analysis at the EPA laboratory in Mississippi. Ground samples were packed tightly in dry ice in coolers and shipped to the EPA laboratory for refrigeration storage until chemical analysis.

Analysis generally followed a modified EPA's Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, with

modifications designed to achieve the lowest possible detection limits. Approximately 30 grams of dried and homogenized feed sample were weighted into an extraction thimble and mixed with anhydrous sodium sulfate. All sample types were fortified with a mixture containing each of the 17, ¹³C labeled 2,3,7,8,-Cl substituted dioxins/furans and the 12, ¹³C WHO PCBs. The samples were extracted with 75/25 hexane/methylene chloride in a soxhlet for 24 hours. All 12 of the WHO dioxin-like coplanar PCBs were evaluated in this project for most of the samples; a small subset only measured 7 coplanar PCBs. Limits of detection (LOD) for dioxins and furans (CDD/Fs) ranged from 0.01 pg/g for the lower chlorinated congeners to 0.20 pg/g for OCDD, and LODs for the polychlorinated biphenyls (PCBs) ranged from 0.01 pg/g for PCB 169, 0.02 pg/g for the most toxic PCB congener, PCB 126, to 4.5 pg/g for PCB 118. Further details on EPA's methods for CDD/F/PCBs are found in Ferrario, et. al.^{6,7}

Results

A total of 87 study samples have been completed, and these include the total mixed ration (TMR) samples from 9 of 10 locations (one location did not supply a TMR sample), all principle forages and concentrates from the 10 locations, and a small number of minor components. A total of 43 samples of minor components from the 10 locations, and one sample each of pooled milk and feces from each location, remain to be analyzed. CDD/F/PCB congener and CDD/F homologue group concentrations were measured. Toxic equivalent (TEQ) concentrations, and average congener concentrations, were derived for both the conditions of ND = 0 and ND = $\frac{1}{2}$ LOD, and using the WHO 1998 TEFs. For all results, the averages and TEQ concentrations were marginally different at ND = 0 and ND = $\frac{1}{2}$ LOD, so all results will be displayed at ND = $\frac{1}{2}$ LOD with no further discussion.

The study has been successful in its principal purpose: to determine the relative contribution of the major feed components to the total dioxin content of the dairy feeds. The results confirm that fodder is the major contributor to the dioxin content of dairy feeds. Since fodders are primarily "leafy" vegetations, as discussed below, this confirms the prevailing hypothesis – that an air-to-leaf pathway is the primary pathway of dioxin exposure for lactating cattle. Key findings include:

- 1) Total Mixed Ration TEQ: The CDD/F/PCB TEQ concentrations of the TMR were all very low, all less than 0.10 pg TEQ/g (ppt) dry weight (dw), and 6 of the 9 TMR samples in fact were less than or equal to 0.05 ppt TEQ dw. Station-specific TMR TEQs are shown in Table 1. This is lower than the diary feeds that were measured in the earlier mass balance study on lactating cows, where CDD/F TEQ concentrations ranged from 0.13 to 0.22 ppt dw². By contrast, vegetation sample concentrations from the 1990s in the US and Europe have been reported to be closer to 1.0 ppt TEQ dw and even as high as several ppt TEQ dw. The highest TMR concentration was 0.09 ppt TEQ dw from Michigan. The contribution to TEQ by CDD/Fs was higher than from PCBs, by about a factor of 2.
- 2) Leafy Vegetation vs. Non-Leafy Vegetation: Research has shown that dioxins sorb to outer portions of vegetation with very little within-plant translocation. For that reason, vegetation which is leafy with a higher surface area to volume ratio, such as grasses, would have higher concentrations as compared to non-leafy vegetative components, such as grains or seeds. To see if that trend held true for these data, the samples were broken into two groups: 35 samples of "leafy" vegetation which included corn silage, alfalfa (hay, silage), sorghum silage, and grass (Bermuda, hay), and 39 samples of "non-leafy" vegetation which included products derived from corn (gluten, ground, meal), soybean (meal, roasted), and cottonseed (whole, hulls), and dried pulps derived from citrus and beets. average concentration of these two groups is shown in columns 2 and 3 of Table 2. It is seen that this trend holds true, with leafy vegetation congener and TEQ concentrations being from about 2 to over 10 times higher than non-leafy vegetation concentrations. This trend was also examined by grouping feed components into the major vegetation types just identified. CDD/F/PCB TEQ concentrations are displayed as bar graphs in Figure 2, which also shows the number of samples in each group. TEQ concentrations range from 0.02 to 0.15 ppt TEQ, with the highest concentrations being the leafy vegetation of alfalfa and grass, and the lowest being the cottonseed and pulp products.
- 3) Predicting a Total Mixed Ration (TMR) Concentration from the Feed Component Concentrations: In theory, one should be able to predict the TMR concentration by measuring the concentrations of the components and then deriving a weighted average concentration. This was attempted for 9 of the 10 sites which had a TMR sample, and Table 2 demonstrates this by showing the concentrations of the major feed components from one of the sites, Michigan, along with the measured TMR concentrations as well as concentrations that could

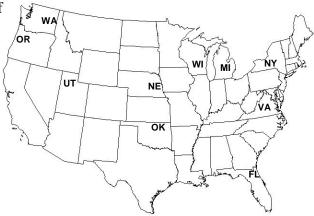
be calculated as weighted averages from the components. The trends shown for Michigan in Table 2 held for all the sites. First, there was a positive association between measured and calculated concentrations - the highest calculated concentrations were also found as the highest in the measured TMR. Second, generally the PCB congener concentrations were higher in the components as compared to the TMR, by about a factor of 1.5 to 2.0 on the average. This is seen particularly for PCB congeners 105 and 118. Similar trends was found in the earlier mass balance study on dairy feeds, where the TEQ concentrations of the feed components were higher than the total mixed feed concentrations. ¹

4) Portion of TMR TEQ Due to "Leafy" Versus "Non-Leafy" Vegetation: "Leafy" vegetation comprises about 76% of the total dry weight of the TMR in Michigan, but these components contribute 94% of the TEQ to TMR. These results can be derived using the percentages of TMR by weight of each component, in combination with the concentration of each component. Similar calculations for the other nine locations – percent of TMR made up of leafy vegetation by weight, and percent of TMR TEQ contributed by leafy vegetation, are: NY – 76, 57; VA – 82, 87; FL – 71, 71; OK – 58, 92; NE – 48, 58; UT – 50, 90; WA – 58; 87; OR – 68, 87; WI – 75, 86. In only one site, NY, did the leafy vegetation contribute more proportionally to weight (76%) than to TEQ (57%). For all other sites, leafy vegetation contributed proportionally more to TEQ as compared to TMR weight; some by nearly a factor of 2. Over all 10 locations, leafy vegetation made up, on average, 66% of TMR by weight, and made up, on average, 81% of TEQ.

References

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Figure 1. Map showing locations of 10 research facilities where dairy feed samples were taken.



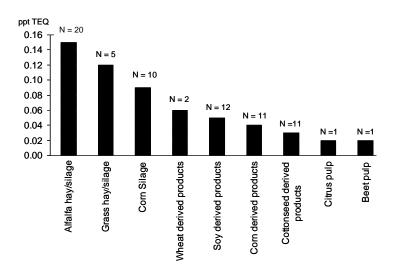


Figure 2. Bar graph showing TEQ concentrations of major groupings of dairy feed components.

Table 1. Feed components for each of 10 sites, percentage of each component in total mixed ration (TMR), and the TEQ concentration of TMR from each site (NA = not available).

Ingredient	NY	VA	FL	MI	WI	OK	NE	UT	WA	OR
I Forgas % by waight								-	-	
corn silage	50	73	42	34	33		18	20		31
alfalfa silage/hay	19	8	5	34	44	13	30	30	60	12
grass hay	3					5				26
sorghum silage			19			36				
II. Non-Forages, % by weight										
soybean (meal, roasted)	8	4	11	6	6	10		2		3
cottonseed (hulls, whole)		3	3	5		3	10	8	8	6
beet (b)/ citrus (c) pulp			6 (c)					7 (b)		
corn (ground, meal, gluten)	17	6	11	11	15	25	40	16		
barley		6								19
molasses								1		
wheat						4			5	
pelleted grain mix									27	
III. Minor Components, % by	weight									
minerals/vitamins	<1	<1	2	4						3
blood meal					<1					
limestone	<1	<1			<1					
urea										
yeast			<1			2				
animal fat additives	<1			<1		<1		<1		
sodium bicarbonate	<1	<1	<1							
concentrate mix							2	14		
other minor	<1			6	<1		<1	2		
TMR TEQ, CDD/F/PCB pg/g	0.05	0.04	0.02	0.09	NA	0.06	0.03	0.04	0.07	0.05

Key: NY = Cornell University, New York; VA = Virginia Tech University; FL = University of Florida; MI = Michigan State University; WI = USDA Dairy Forage Research Center, Wisconsin; OK = Oklahoma State University; NE = University of Nebraska; UT= Utah State University; WA = Washington State University; OR = Oregon State University

Table 2.	Sample result	concentrations in	ppt dry wt.	See table notes below.
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Congener	Vege	tation	Feed Components For Michigan (MI) Site							MI - TMR		
	Leafy	Non- leafy	CS1	CS2	AH1	AH2	АН3	CG	SM	CW	Meas	Calc
PCB 77 PCB 81 PCB 105 PCB 114 PCB 118 PCB 123 PCB 126 PCB 156 PCB 157 PCB 167 PCB 169 PCB 189	2.51 9.03 20.74 0.24 1.58 0.39 0.02	0.59 3.00 7.95 0.03 0.84 0.17 0.01	1.34 6.34 15.77 0.19 0.77 0.25 0.25	1.68 12.82 30.37 0.35 3.75 0.97 0.97	2.56 0.12 10.14 0.41 19.41 0.31 0.20 1.80 0.47 0.51 0.47	6.88 0.29 25.29 1.54 59.49 1.16 0.55 3.57 0.89 1.37 0.89 0.24	5.14 0.24 22.65 1.09 50.55 0.68 0.48 3.78 0.97 1.39 0.04 0.25	1.65 0.10 15.78 0.60 29.77 0.45 0.05 4.35 0.89 1.08 0.01 0.07	1.97 0.17 7.79 0.44 17.01 0.29 0.06 3.35 0.70 0.78 0.01 0.11	0.37 0.02 5.05 0.10 8.42 0.09 0.02 2.01 0.43 0.44 0.01	1.55 5.87 16.87 0.25 0.61 0.17 0.02	2.58 12.90 28.68 0.17 2.70 0.66 0.02
2378-D 12378-D 123478-D 123678-D 123789-D 1234678-D OCDD	0.04 0.03 0.04 0.10 0.08 1.63 15.39	<0.01 0.01 0.01 0.02 0.02 0.15 0.92	0.01 004 0.04 0.08 0.08 1.05 5.10	0.01 0.08 0.12 0.24 0.24 4.13 19.69	0.01 0.01 0.01 0.04 0.01 0.20 0.83	0.01 0.04 0.04 0.07 0.04 0.53 2.63	0.01 0.02 0.04 0.08 0.06 0.88 4.57	0.01 0.01 0.01 0.01 0.01 0.02 0.54	0.01 0.01 0.01 0.01 0.01 0.02 1.07	0.01 0.01 0.01 0.01 0.01 0.01 0.14 1.29	0.01 0.01 0.04 0.08 0.07 1.11 7.27	<0.01 0.03 0.04 0.08 0.07 1.12 5.56
2378-F 12378-F 23478-F 123478-F 123678-F 234678-F 123789-F 1234678-F 1234789-F OCDF	0.03 0.02 0.03 0.03 0.03 0.05 0.01 0.40 0.03 0.55	0.01 0.01 0.01 0.01 0.01 0.02 <0.01 0.04 0.01 0.08	0.02 0.01 0.03 0.02 0.04 0.05 0.01 0.48 0.03 0.48	0.03 0.04 0.06 0.11 0.20 0.20 0.01 3.07 0.10 2.65	0.03 0.01 0.01 0.01 0.01 0.06 0.01 0.06 0.02 0.11	0.03 0.02 0.04 0.03 0.04 0.03 0.01 0.20 0.02 0.13	0.04 0.03 0.04 0.04 0.04 0.11 0.01 0.24 0.03 0.26	0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01	0.03 0.01 0.02 0.01 0.01 0.01 0.01 0.01 0.01	0.06 0.05 0.05 0.01 0.01 0.01 0.03 0.02 0.01	0.01 0.01 0.02 0.03 0.03 0.04 <0.01 0.51 0.03	0.03 0.02 0.03 0.03 0.05 0.07 <0.01 0.67 0.03
PCB-TEQ D/F-TEQ	0.03 0.14	<0.01 0.03	0.02 0.10	0.04 0.31	0.02 0.03	0.07 0.11	0.06 0.10	0.01 0.02	0.01 0.03	<0.01 0.05	0.02 0.07	0.03 0.11

Notes

a) "---" means congener not measured in this sample

b) Vegetation: "Leafy" vegetation includes: alfalfa (hay, silage), corn silage, sorghum silage, and grass (hay, bermuda). "Non-leafy" vegetation includes: corn-derived products (gluten, ground, meal), soybean-derived products (meal, roasted), cottonseed (whole, hulls), dried pulp

c) Feed abbreviations including percentage of total mixed ration in MI: CS1 = corn silage from silo 1 (17%), CS2 = corn silage from silo 2 (17%), AH1 = alfalfa hay from silo 1 (6%), AH2 = alfalfa hay from silo 2 (9%), AH3 = alfalfa hay from silo 3 (19%) CG = corn, ground (11%), SM = soybean meal (6%), CW = cottonseed, whole (6%)

d) MI = Michigan; TMR = total mixed ration; "meas" = measured, "calc" = weighted average calculated TMR concentration